MINNESOTA CHROMATOGRAPHY FORUM

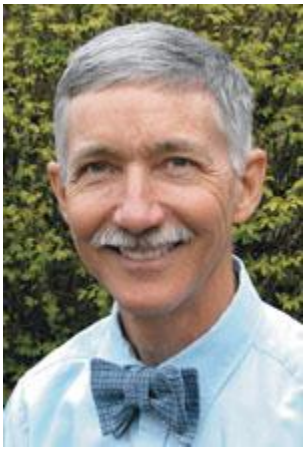
Making HPLC Methods Work

by John Dolan

This course is designed for laboratory workers who are responsible for running HPLC methods on a day-to-day basis. No prior experience is needed, although those with some laboratory experience will certainly benefit more than those with no experience at all. New workers in the lab, scientists from other disciplines that have to use HPLC as a tool, laboratory managers, and quality assurance staff will all benefit from this course. This class focuses on existing HPLC methods and how to keep them running reliably. It includes a look at how the hardware operates, how reversed- and normal-phase separations work, and troubleshooting separation problems. You will gain an understanding about what happens "behind the scenes" in an HPLC method. Armed with this understanding of the equipment and separations, you will be more able to keep methods working reliably and getting them back to operation when problems occur. You will understand some of the quality-related practices that can be used to improve the reliability and usefulness of the data you gather.

Course Outline

I. Introduction and HPLC Basics
   a. System suitability
   b. Basic HPLC processes
   c. Key chromatographic measurements
II. HPLC Equipment
   a. Reservoirs & mobile phases
   b. Pumps & mixing
   c. Tubing & fittings
   d. Injectors & autosamplers
   e. Detectors
III. HPLC Columns
   a. The underlying silica
   b. Bonded phases
   c. Care & treatment of columns
IV. Reversed-Phase HPLC
   a. Retention mechanism
   b. The role of the solvent
   c. The role of the bonded phase
   d. Other variables: pH, temperature, ion pairing
V. Normal-Phase and HILIC
   a. Retention fundamentals
   b. Solvents
   c. HILIC (hydrophilic interaction chromatography)
VI. Troubleshooting HPLC Separations
   a. Why columns die
   b. Physical problems with the column
   c. Chemical problems with the column
   d. Non-column problems
VII. Quality Issues
   a. Validation
   b. Regulatory issues
   c. System suitability
   d. Improving precision
   e. Method adjustment vs. method change



Dr. John Dolan is a Principal Trainer and consultant for LC Resources, Inc.. John received his Ph.D. from the University of California at Davis in 1976 and has more than 30 years of HPLC experience. After finishing graduate school, he did postdoctoral work at Northeastern University and then joined Technicon Instruments Corporation, where he worked for three years developing clinical HPLC technology. He moved to IBM Instruments, where he was involved in design and support of LC, IR, and UV products. As a columnist for LC/GC magazine, he has written over 300 installments of the "LC Troubleshooting" monthly column since 1983. In 1984, John and Lloyd Snyder founded LC Resources, which offered support to the separations community via teaching, software, consulting, and laboratory services. In 2002, LC Resources sold its software products to Rheodyne, the laboratory to Bioanalytical Systems, and retained the training business. After acting as General Manager of the BASi Northwest Laboratory for three years, John now spends full time teaching and consulting. He has written more than 100 scientific papers on LC theory, instrumentation, and applications as well as a book on troubleshooting LC instruments and methods. John is the 2002 recipient of the MCF Palmer Award.